

2020

An Evaluation of Sonication Pretreatment for Enhancing Saccharification of Brewers' Spent Grain


Shady Hassan

Rajeev Ravindran

Swarna Jaiswal

See next page for additional authors

Follow this and additional works at: <https://arrow.tudublin.ie/tfschafart>

 Part of the [Food Biotechnology Commons](#), [Food Chemistry Commons](#), [Food Microbiology Commons](#), and the [Food Processing Commons](#)

This Article is brought to you for free and open access by the School of Culinary Arts and Food Technology at ARROW@TU Dublin. It has been accepted for inclusion in Articles by an authorized administrator of ARROW@TU Dublin. For more information, please contact yvonne.desmond@tudublin.ie, arrow.admin@tudublin.ie, brian.widdis@tudublin.ie, aisling.coyne@tudublin.ie, fiona.x.farrell@tudublin.ie.



This work is licensed under a [Creative Commons Attribution-Noncommercial-Share Alike 3.0 License](#)

Authors

Shady Hassan, Rajeev Ravindran, Swarna Jaiswal, Brijesh K. Tiwari, and Gwilym A. Williams



An evaluation of sonication pretreatment for enhancing saccharification of brewers' spent grain

Shady S. Hassan^{a,c}, Rajeev Ravindran^{a,c}, Swarna Jaiswal^a, Brijesh K. Tiwari^b, Gwilym A. Williams^c, Amit K. Jaiswal^{a,*}

^a School of Food Science and Environmental Health, College of Sciences and Health, Technological University Dublin-City Campus, Cathal Brugha Street, Dublin 1, Ireland

^b Department of Food Chemistry & Technology, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

^c School of Biological Sciences and Health Sciences, College of Sciences and Health, Technological University Dublin-City Campus, Kevin Street, Dublin 8, Ireland

ARTICLE INFO

Article history:

Received 17 September 2019

Revised 17 January 2020

Accepted 10 February 2020

Available online 20 February 2020

Keywords:

Brewer's spent grain

Pretreatment

Ultrasound

Ethanol

ABSTRACT

This paper deals with the investigation of ultrasound (US) pretreatment of brewer's spent grain (BSG) as a means of releasing fermentable sugars, and the subsequent production of ethanol from this lignocellulosic biomass. Using response surface methodology (RSM), the influence of US power, time, temperature and biomass loading on fermentable sugar yield from BSG was studied. The optimal conditions were found to be 20% US power, 60 min, 26.3 °C, and 17.3% w/v of biomass in water. Under these conditions, an approximate 2.1-fold increase in reducing sugar yield (325 ± 6 mg/g of biomass) was achieved, relative to untreated BSG (151.1 ± 10 mg/g of biomass). In contrast to acid or alkaline pretreatment approaches, the use of water obviated the need for neutralization for the recovery of sugars. The characterization of native and pretreated BSG was performed by HPLC, FTIR, SEM and DSC. Fermentation studies using *S. cerevisiae* growing on pretreated BSG resulted in a conversion of 66% of the total sugar content into ethanol with an ethanol content of 17.73 ± 2 g/100 g of pretreated BSG. These results suggest that ultrasound pretreatment is a promising technology for increased valorization of BSG as a feedstock for production of bioethanol, and points to the need for further work in this area.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

According to a Brewers of Europe report published in 2017, Europe is the second largest beer producer in the world after China, with over 8490 breweries producing over 400,168 million hectoliters of beer in 2016 (BOE, 2017). Brewers' spent grain (BSG) is the most plentiful agro-industrial waste generated from the beer-brewing process, and about 3.4 million tonnes are produced annually in the EU (McCarthy et al., 2013). Spent grain is the insoluble part of the barley grain, separated during the mashing process before fermentation of the soluble liquid wort (Lynch et al., 2016). BSG represents about 85% of the total by-products, with about 2 Kg generated per 10 L of beer. BSG composition offers considerable options for developing value-added products, but the complex structure of lignin, and the high crystallinity of cellulose, reduces its accessibility to hydrolytic enzymes. This structure necessitates a pretreatment step prior to enzymatic hydrolysis.

Significantly, the EU aims to replace 10% of all transport fuel with biofuel by 2020. Such targets are an impetus to the investigation of the lignocellulosic waste utilization in order to achieve sustainable and profitable production of bioethanol. The current biofuel market is sensitive to sugar prices and supply shortages.

Previous studies have employed BSG for production of ethanol (sake) at 37 g/L over a 10 day fermentation using *S. cerevisiae* and *A. oryzae* (Wilkinson et al., 2017). Acid pre-treatment of BSG is a common approach in the literature. Plaza et al. (2017) pretreated BSG with sulfuric acid (15% w/w BSG, pH 1.0, 121 °C), producing 75 g butanol/kg BSG. Rojas-Chamorro et al. (2018) optimized the phosphoric acid pretreatment for BSG, and reported recovery of 92% of total sugars from this substrate under harsh of 155 °C and 2.0% H₃PO₄; the authors also reported conversion of 69% of the total sugars in the BSG to ethanol using *Escherichia coli*. Such conventional methods for pretreatment have major drawbacks, including high energy consumption, the necessity to use harsh chemicals, production of inhibitors or low efficiency in large scale production (Hassan et al., 2018a).

The application of ultrasound technology for pretreatment of lignocellulosic biomass holds some potential for large scale processes (Hassan et al., 2018a). Ultrasonic (US) pretreatment in liquid

* Corresponding author.

E-mail addresses: amit.jaiswal@TUDublin.ie, akjaiswal@outlook.com (A.K. Jaiswal).

media results in cavitation phenomena, whereby microbubbles are formed which grow and then violently collapse at a critical size, converting sonic into mechanical energy. Such cavitation effects generate high temperature, pressure and violent shear forces, which lead to the formation of the 'hot-spot' effect in the liquid, and the generation of free radicals (Bundhoo and Mohee, 2018). Such effects may be postulated to aid in disruption of the lignocellulosic complex structure, decreasing the degree of crystallinity of the cellulose, and increasing the accessible surface area to improve enzymatic hydrolysis (Suresh et al., 2014).

Recently, chemical pretreatment methods combined with US technique have received some attention (Wang et al., 2018). However, very few reports discuss the ultrasound pretreatment of lignocellulosic material using water as solvent, and optimization of such processes has not been attempted. He et al. (2017) compared the ultrasound pretreatment (300 W, frequency of 28 kHz) of wood in soda solution, acetic solution, and distilled water. The authors reported an increase in sample crystallinity up to 35.3% in the case of soda solution, and up to 35.5% in the case of distilled water or acetic solution, which was due to physico-chemical-induced structural changes. However, the use of only water as a matrix for treatment, as opposed to conventional acid and alkaline approaches would eliminate the use of harsh chemicals, and the consequent problems with waste treatment.

The critical parameters for optimization of an ultrasound-mediated process for biomass pretreatment include US power (Song et al., 2015), biomass loading (Sasmal et al., 2012), treatment time, (Rehman et al., 2014; Sasmal et al., 2012; Song et al., 2015) and temperature (Rehman et al., 2014). Optimization of the pretreatment process by one-factor at a time in a multifactorial process is time-consuming and does not consider the possible interactive effects between the variables. Such limitation can be overcome by using Response Surface Methodology (RSM) as a means of statistical modeling and optimization of the multiple variables of pretreatment processes (Flores-Gómez et al., 2018).

The aim of the present study is to investigate the effect of US pretreatment on brewers' spent grain (BSG) in an aqueous medium with the goal of achieving maximum cellulose (glucose) and hemicellulose (xylose) recovery for bioethanol production, and maximum lignin recovery for use in a sugar-lignin platform biorefinery (Hassan et al., 2018b, 2019).

2. Material and methods

2.1. Materials

Brewer's spent grain (BSG) was generously donated by a local brewery in Dublin, Ireland. The BSG was dried at 60 °C for 48 h followed by milling and sorting using a 350 µm sieve. The BSG was then stored in a dry place at ambient temperature for further experiments. Cellulase from *Trichoderma reesei* (aqueous solution, ≥700 units/g), and hemicellulase from *Aspergillus niger* (powder, 0.3–3.0 unit/mg solid) were purchased from Sigma Aldrich (Ireland), and were of analytical grade. The ethanol producing yeast, *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (ATCC® 9763™), was obtained from the TU Dublin microbiology repository. The culture was grown in slants at 30 °C for 24 h in Yeast Peptone Dextrose (YPD) medium.

2.2. Methods

2.2.1. Compositional analysis

Compositional analysis of BSG, before and after pretreatment, was carried out according to the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al., 2008). In this method, BSG

samples are subjected to acid hydrolysis using 72% (v/v) H₂SO₄ at 30 °C for 60 min, followed by a second step of acid hydrolysis by 4% (v/v) H₂SO₄, and then autoclaving at 121 °C for 60 min. Post hydrolysis, the mixture was separated using a filtration crucible and dried at 105 °C until constant weight was achieved. The dried solids were then ashed at 595 °C for 24 h in a blast furnace to calculate the acid insoluble lignin (AIL) content, while the acid soluble lignin (ASL) fraction in the liquid was determined using UV-Vis absorbance at 205 nm. Total lignin content in each sample was calculated by combining the value of AIL and ASL. Furthermore, the reducing sugar concentration in the liquid was estimated by the dinitrosalicylic acid (DNS) method (Miller, 1959), while the detection and quantification of monosaccharides were performed by an Alliance® HPLC system (Waters, e2695 Separation module) using a Rezex ROA-Organic Acid H⁺(8%) column, (350 × 7.8 mm; Phenomenex, UK) with 5 mM H₂SO₄ as the mobile phase at 65 °C, maintaining a flow rate of 0.6 mL/min. The HPLC system was equipped with a refractive index detector for the detection of monosaccharides.

2.2.2. Experimental design

For experimental design and optimization of ultrasound pretreatment of BSG, the Response Surface Methodology (RSM) based on Central Composite Design (CCD) was employed (Manorach et al., 2015). The effects of four pretreatment variables, each at five levels, on total sugar yield from BSG after saccharification were examined: biomass loading, temperature, ultrasound power and time. (Table 1). This used a CCD with five replicates at the center points, requiring 30 experiments. Experimental runs were randomized in order and carried out in triplicate. These pretreatment variables were further optimized using RSM. Experimental design was carried out using STATGRAPHICS Centurion XV software (StatPoint Technologies Inc. Warrenton, VA, USA).

2.2.3. Pretreatments

A total of 30 experiments as given by the experimental design were carried out to investigate the effect of varying conditions of ultrasound pretreatment on the enzymic hydrolysis of BSG (Fisher Scientific TI-H-10 Ultrasonic bath, output 750 W). After pretreatment, the biomass was collected, air-dried, and stored for further enzymatic hydrolysis, compositional analysis and characterization.

2.2.4. Enzymatic hydrolysis

To perform the saccharification, 1 g of BSG, 158.76 µL of cellulase (77 FPU/mL), and 153.3 µL hemicellulase (72 U/ mL) were mixed in sodium citrate buffer (0.05 M) and distilled water at pH 5.4 (total volume 10 mL) and heated to 50 °C for 120 h (Ravindran et al., 2018). After treatment, the hydrolysate liquors were collected and stored at 4 °C for further compositional analysis.

2.2.5. Model development and optimization

After conducting the experiments, a second-order polynomial regression model, as given by Eq. (1), was generated and analysed by the statistical software STATGRAPHICS Centurion XV software (StatPoint Technologies Inc. Warrenton, VA, USA) to define the response in terms of the independent variables.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \quad (1)$$

where Y, β_0 , β_i , β_j , β_{ij} , X represent process response (total sugar yield), linear coefficients, quadratic coefficients, interaction coefficients and coded independent variables (biomass loading, temperature, ultrasound power, and time), respectively. The regressors (β_0 , β_i , β_j , and β_{ij}) provide a quantitative measure of the significance of linear effects, quadratic of factors and interactions between factors.

Table 1
Experimental range and levels of the independent variables.

Code	Variables	Range and levels				
		–2	–1	0	+1	+2
A	Solids/Liquids ratio (g-dry/mL, %)	5	10	15	20	25
B	Temperature (°C)	20	30	40	50	60
C	Ultrasound Power (%)	20	40	60	80	100
D	Time (min)	20	30	40	50	60

The significant differences between each pretreatment with respect to the components of BSG were analyzed by performing analysis of variance (ANOVA) and multiple comparisons (Fischer's least significant difference test); Values of $p < 0.05$ was considered as significant.

2.2.6. Fermentation

BSG hydrolysate (supplemented with all yeast extract peptone dextrosebroth medium ingredients except dextrose) was used for bioethanol fermentation as described by Kumar et al., 2011. A 24 h cell suspension of *Saccharomyces cerevisiae* (10^6 cfu/ml) was grown in shake flask culture (1.0 L, 50% working volume) at 30 °C for 72 hrs. Gas Chromatography (Bruker Scion 456-GC coupled with flame ionization detector) was used to quantify the ethanol produced. Temperature of the injector, the detector and column oven were 220 °C, 200 °C and 160 °C, respectively. The column oven initial temperature was 80 °C and was set to increase temperature by 40 °C/min and held for 7 min until the target temperature was achieved (Ravindran et al., 2017b).

2.2.7. Characterization

2.2.7.1. Scanning electron microscopy. The morphological structure of BSG before and after pretreatment was observed by performing field emission-scanning electron microscopy FE-SEM (Hitachi SU-70). Dried samples of the untreated and pretreated BSG were subjected to FE-SEM at an electron beam energy of 0.5 keV (Raghavi et al., 2016).

2.2.7.2. FTIR analysis. FTIR analysis was performed to observe any alteration in the composition of the BSG by assessing possible changes in functional groups after pretreatment and compared with native BSG. A Perkin Elmer Spectrum GX FTIR (UATR) Microscope (USA) was used for this part of the work (Raghavi et al., 2016). The FTIR spectra were recorded from 4000 to 400 cm^{-1} , with 32 scans at a resolution of 0.3 cm^{-1} in transmission mode.

2.2.7.3. Thermal behavior. The thermal behavior of BSG before and after pretreatment was studied and compared using differential scanning calorimetry (DSC). The thermal analysis instrument (Shimadzu DSC-60) was controlled by TA-60WS software. To perform the analysis, 55 mg of each BSG sample was placed in an aluminum pan, with an empty pan serving as a reference. The measurements were carried out between 25 °C and 500 °C, with a linear increase of 10 °C per min (Ballesteros et al., 2014).

3. Results and discussion

3.1. Compositional analysis

Polysaccharides were found to represent up to 53% of the dry weight of BSG, comprising xylan (38%), glucan (15%), and lignin (10%). This is largely in agreement with the values previously reported in the literature for this biomass type (Lynch et al., 2016). The hemicellulose and cellulose content of BSG as described in the literature varies between 19 and 40 % and 9–25% per dry

matter, respectively (Xiros et al., 2008). This notwithstanding, the chemical composition of BSG is known to vary with barley cultivars, cultivation conditions, harvest time, malting practices, mashing regime, the presence of hops or adjuncts, and brewing conditions associated with lager and ale fermentations (Mussatto, 2014).

3.2. Modeling and optimization of ultrasound pretreatment

The total sugar yield from enzymatic hydrolysis was used in response surface methodology (RSM) to optimize the ultrasound-assisted pretreatment. The model adequacy was confirmed based on the coefficient of determination (R^2) and adjusted coefficient of determination (R^2 -adj). Accuracy range for R^2 is from 0 to 1, considering that a value closer to 1 means that the model is more accurate. A value of 98.28% R^2 was observed in the present work, while R^2 -adj was 96.68%, illustrating that the model adequately fits the data. The polynomial equation (2) describing the total sugar yield behavior is:

$$\begin{aligned} \text{Reducing sugar (mg/ml)} = & 2.18129 - 0.1184 \times A \\ & - 0.009875 \times B - 0.0658167 \\ & \times C + 0.135458 \times D \\ & - 0.00243 \times A^2 + 0.0003025 \\ & \times A \times B + 0.0012275 \times A \\ & \times C + 0.002905 \times A \times D \\ & - 0.00053875 \times B^2 \\ & + 0.00132438 \times B \times C \\ & - 0.00117125 \times B \times D \\ & + 0.000285937 \times C^2 \\ & - 0.00109875 \times C \times D \\ & - 0.0008125 \times D^2 \end{aligned} \quad (2)$$

The significance of the coefficients of the model was determined by ANOVA. The ANOVA table showed that 12 effects have P-values less than 0.05, indicating that they are significantly different from zero at the 95.0% confidence level, and implying a considerable effect of these coefficients on reducing sugar yield. The predicted levels of sugar yield in pretreated BSG using the Eq. (2) are given in Table 2, along with experimental data.

Additionally, response surface plots were generated as a function of two-factors-at-a-time in order to understand the principle interaction effects of different variables, and to determine the optimal level of each variable for maximum response (Fig. 1). A maximum reducing yield was observed with low ultrasound power (20%) and temperature (26 °C), as well as high biomass loading (17 g/100 mL) and long pretreatment time (60 min). Under optimum conditions, the model predicted the maximum sugar yield to be 388 mg/mL of reaction volume.

After the optimized pretreatment of the native BSG, and following saccharification, 74% of sugars in BSG were recovered, while no degradation of lignin was observed (remaining as 10.6 g per 100 g

Table 2

Experimental conditions and results of central composite design.

Run	Variables				Response	
	Factor A SL ratio (%)	Factor B Temp (°C)	Factor C Ultrasound power (%)	Factor D Time (min)	Reducing Sugar (mg/mL) Experimental	Predicted
1	20	30	80	50	139	144
2	5	40	60	40	97	115
3	15	40	60	40	150	150
4	15	40	60	60	118	120
5	20	30	40	30	132	134
6	10	30	40	30	185	180
7	15	60	60	40	93	97
8	15	40	60	40	150	150
9	15	40	60	40	150	150
10	10	50	40	30	123	116
11	15	40	100	40	177	179
12	15	40	20	40	208	212
13	10	30	80	50	89	83
14	15	40	60	20	111	115
15	15	40	60	40	150	150
16	10	50	80	50	82	78
17	15	40	60	40	150	150
18	10	50	40	50	115	110
19	10	50	80	30	177	172
20	20	50	40	50	126	128
21	25	40	60	40	147	136
22	20	50	80	30	179	181
23	20	30	80	30	133	133
24	10	30	40	50	225	221
25	20	50	40	30	74	76
26	10	30	80	30	135	130
27	20	50	80	50	145	145
28	20	30	40	50	233	233
29	15	40	60	40	150	150
30	15	20	60	40	158	160

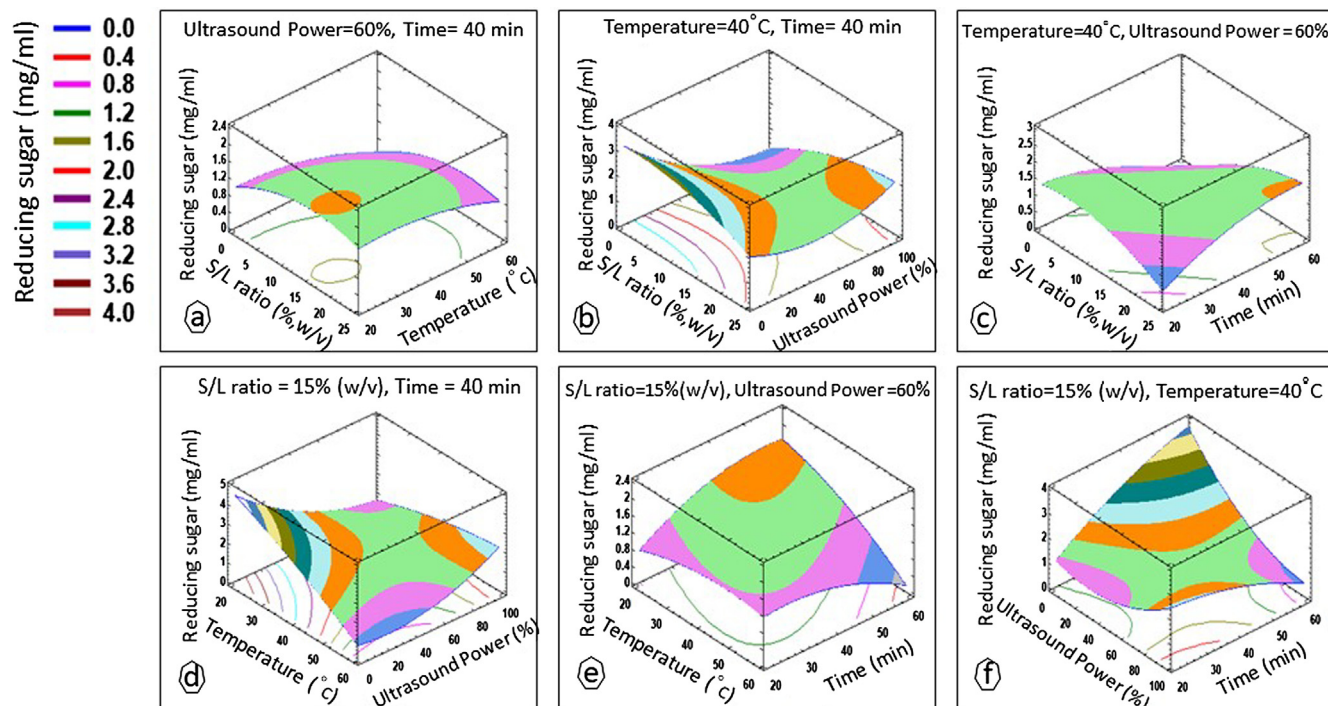


Fig. 1. Response surface plots representing the effect of independent variables on reducing sugar yield: (a) Effect of solid-to-liquid ratio and temperature on reducing sugar yield when the response surface is fixed at ultrasound power = 60% and time = 40 min; (b) Effect of solid-to-liquid ratio and ultrasound power on reducing sugar yield when the response surface is fixed at temperature = 40 °C and time = 40 min; (c) Effect of solid-to-liquid ratio and time on reducing sugar yield when the response surface is fixed at temperature = 40 °C and ultrasound power = 60%; (d) Effect of temperature and ultrasound power on reducing sugar yield when the response surface is fixed at Solid-to-liquid ratio = 15% (w/v) and time = 40 min; (e) Effect of temperature and time on reducing sugar yield when the response surface is fixed at solid-to-liquid ratio = 15% (w/v) and ultrasound power = 60%; and (f) Effect of ultrasound power and time on reducing sugar yield when the response surface is fixed at solid-to-liquid ratio = 15% (w/v) and temperature = 40 °C.

of raw BSG). Improvement in saccharification of BSG without lignin degradation may be attributed to the type of solvent used in this pretreatment step (water, with no chemicals), and the mechano-acoustic (physical) effects of ultrasound that increase surface erosion and pore size, and therefore the accessibility of biomass to hydrolytic enzymes (Bussemaker and Zhang, 2013). Obtaining high saccharification yields from lignocellulose, while maintaining a high amount of lignin after the pretreatment available for further valorization, can maximize the utilization of lignocellulose.

For the validation of the model, a confirmation experiment was conducted using the optimized parameters, and the experimentally obtained values of total reducing sugar amounted to 325 ± 1.0 mg/mL. Thus, since the experimentally obtained values of total reducing sugar from native BSG amounted to 151 ± 0.6 mg/mL, this model provided a 2.1-fold higher reducing sugar yield.

Ultrasonic frequency has a significant effect on ultrasound pretreatment, due to its influence on the critical size of the cavitation bubble. Lower frequency ultrasound (20–100 kHz) can produce more violent cavitation, resulting in higher localized temperatures and pressures at the cavitation site, as well as more effective shock waves (Terán Hilaes et al., 2018); water molecules dissociate into oxidative radicals that oxidize and degrade organic molecules. Therefore, low frequency ultrasound is commonly used in biomass processing for intense physical effects, such as cell disruption (Tang and Sivakumar, 2015). Using similar low frequency ultrasound for pretreatment of biomass was reported by other researchers (Rehman et al., 2014) who used ultrasonication at an operating frequency of 20 kHz (power 750 W) and employing 20% of amplitude for enhancing sulfuric acid pretreatment of rice straw. Moreover, the results were consistent with reported data in the literature (Subhedar and Gogate, 2013) for newspaper pretreated with ultrasound (at frequency of 20 KHz) combined with alkali

(NaOH) solution (concentration of 1 N). Eblaghi et al. (2016) also employed ultrasonic irradiation at a frequency of 35 kHz combined with alkaline solution (3% NaOH concentration) for pretreatment of sugarcane bagasse.

The ultrasonic treatment is also known to be influenced by solvent properties (Bussemaker and Zhang, 2013): water can produce more favorable conditions for cavitation than solvents with higher viscosity, surface tension or density. According to the literature, increases in biomass loading, temperature, sonication time, or the US power applied to the reaction mixture results in an increased rate of the reaction only up to a certain point; further increases in these parameters do not result in greater disruption effects (Bussemaker and Zhang, 2013; Karimi et al., 2014).

3.3. Fermentation

The hydrolysate obtained after enzymatic digestion of the ultrasound pretreated BSG (under the optimum conditions) was used for bioethanol production using *S. cerevisiae*, and gas chromatography was used to quantify the ethanol produced. An ethanol content of 17.73 ± 2 g/ 100 g of pretreated BSG was obtained after fermentation, compared to 11.53 ± 1.1 g/100 g for native BSG. The ethanol yield from this study was 88% of the theoretical yield (0.51 g ethanol/g sugars). Table 3 summarizes the findings of various authors regarding the production of bioethanol from BSG using different pretreatments. Ethanol production from ultrasound-pretreated BSG in this study is higher than that reported in earlier work using acid-pretreated BSG (15 g and 10.3 g / 100 g BSG; as reported by Rojas-Chamorro et al., 2017, and Raftopoulou, 2019, respectively.), while being similar to that obtained by recent research on BSG (17.9–18 g of ethanol per 100 g of BSG) using optimized chemical pretreatment (Rojas-Chamorro et al., 2018; and Rojas-Chamorro

Table 3
Comparison of bioethanol production by fermentation of BSG from various studies.

Pretreatment	Conditions	Post-treatment	Culture	Yield	Reference
Ultrasound	20% power, 60 min, 26.3 °C, and 17.3% w/v of biomass in water.	No	<i>S. cerevisiae</i>	17.73 g/100 g BSG	This study
Alkaline	44.5 °C, 154 min, and 107 mg (NaOH) /g BSG	H ₂ SO ₄	* <i>Zymomonas mobilis</i> 8b	18.8 g/100 g BSG	Raftopoulou, 2019
Acid	95 °C, 46 min, and 168 mg (H ₂ SO ₄) /g BSG	NaOH	* <i>Zymomonas mobilis</i> 8b	10.3 g/100 g BSG	Raftopoulou, 2019
Dilute phosphoric acid	25% BSG solid load, 130 °C, 1% (v/v) H ₂ SO ₄ , for 26 min.	No	* <i>Escherichia coli</i> SL100	18 g/100 g BSG	Rojas-Chamorro et al., 2019
Dilute phosphoric acid	BSG with a solid/liquid ratio of 1:8 (w/v), aqueous phosphoric acid solution 2% (w/v) at 155 °C.	Washed with distilled water	* <i>Escherichia coli</i> SL100	17.9 g/ 100 g BSG	Rojas-Chamorro et al., 2018
Dilute phosphoric acid	75 g of BSG, 600 mL of aqueous phosphoric acid solution 2% (w/v) at 155 °C.	Washed with distilled water	<i>S. cerevisiae</i>	15 g/ 100 g BSG	Rojas-Chamorro et al., 2017

* Genetically modified microorganism.

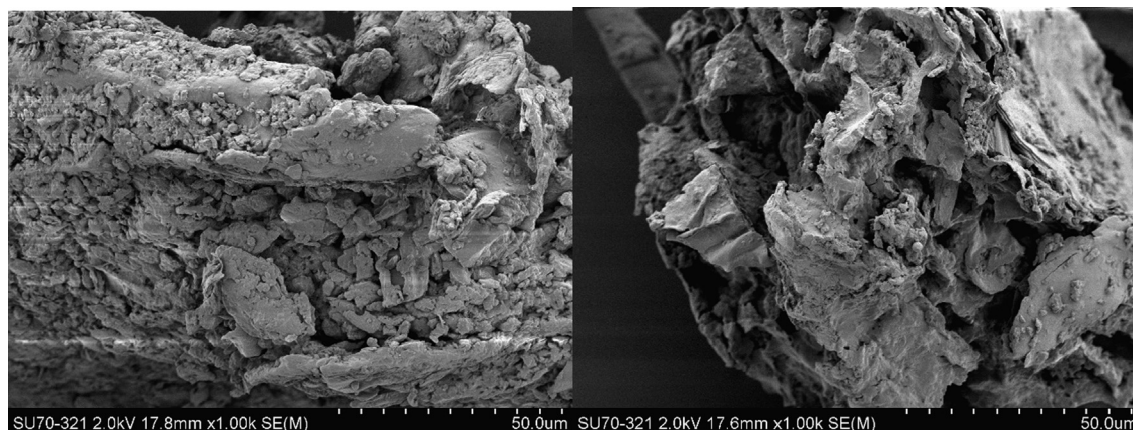


Fig. 2. Micrographs by scanning electron microscopy (SEM) of native (left), and the modified structure of the pretreated BSG (right). Magnification, 1000-fold.

et al., 2019) and 18.8 g/100 g BSG using the recombinant *Zymomonas mobilis* strain 8b (Raftopoulou, 2019). It is also worth pointing out that using simultaneous saccharification fermentation (SSF) instead of the separated hydrolysis fermentation (SHF) may reduce the total time required for ethanol production from BSG as the time for enzymatic hydrolysis is not required (Rojas-Chamorro et al., 2017). However, authors reported that SHF gave highest value of ethanol productivity (0.94 g/L/h after 72 h enzymatic hydrolysis + 24 h fermentation) as compared to SSF (0.595 g/L/h without xylanases after 72 h fermentation, and 0.829 g/L/h with xylanases after 30 h fermentation). This preliminary finding suggests the potential of ultrasound pretreatment in a water solvent system for BSG degradation, thereby dispensing with the need for acid-alkali additions.

3.4. Characterization

3.4.1. Scanning electron microscopy

SEM analysis of the raw (control) and pretreated BSG revealed surface modifications in response to pretreatment (Fig. 2). The untreated BSG had an undulating and crumbled surface as a result of the brewing process. The ultrasound pretreated BSG showed a porous surface of uneven and non-uniform cavities. This may be due to the modifications in the external fibers arising from the effect of the cavitation due to ultrasound waves (Ravindran et al., 2017a). Gabhane et al. (2014) compared alkali-microwave and

alkali-sonication pretreatment of lignocellulosic biomass, and reported that the latter resulted only in fibrillation of the cell wall of the pretreated biomass; however, microwave pretreatment led to complete tissue collapse. Ultrasound pretreatment was found to increase the total surface area of BSG that was exposed to enzyme activity, and enhanced the enzyme accessibility.

3.4.2. FTIR analysis

The chemical changes in the functional groups of the BSG were studied based on FTIR analysis (Fig. 3). The pretreated BSG displayed significant decreases in band intensities at characteristic peaks for cellulose (3290 cm^{-1}), hemicellulose (1030 cm^{-1}) and lignin (1240 cm^{-1}) (Table 4). Similar reduction in intensity of peaks representing functional groups of BSG after ultrasound-assisted pretreatment of lignocellulose were reported by Ravindran et al. (2017a). These observations indicate that ultrasound treatment disturbed the lignocellulosic structure of BSG, increasing the yield of the reducing sugar.

3.4.3. Differential scanning calorimetry

Differential scanning calorimetry determines the difference in the heat flow associated with heating, cooling or isothermal conditions of the sample, compared with a reference, and as a function of temperature. The DSC thermogram (Fig. 4) represented the thermal characteristics of the native and the pretreated BSG between $20\text{ }^{\circ}\text{C}$ and $500\text{ }^{\circ}\text{C}$, which were obtained at a heating rate of $10\text{ }^{\circ}\text{C}/\text{min}$.

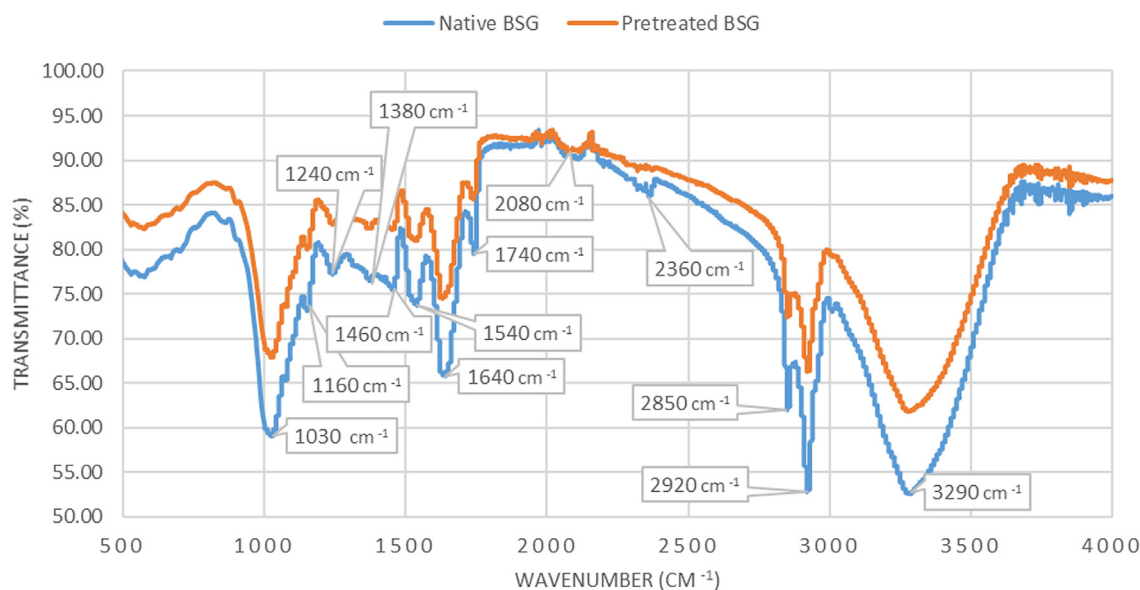


Fig. 3. FTIR spectra of native and pretreated BSG.

Table 4

FTIR peak assignments for lignocellulose.

Wave number (Cm^{-1})	Assignment	References
1030	C–O stretching vibration of hemicelluloses	Peng et al., 2015
1160	C–O stretching vibration of acetyl xylan	Peng et al., 2015
1240	C–O stretching of syringyl lignin	Kalia, 2018
1380	phenolic OH and aliphatic C–H in methyl groups	Coletti et al., 2013
1460	C–H bending vibration of chitosan	Peng et al., 2015
1540	vibration of C=O	Karta et al., 2016
1640	conjugated C=O stretch	Khanna et al., 2017
1740	C=O stretching vibration of acetyl xylan	Peng et al., 2015
2080	vibration of C≡N	Kartel and Galysh, 2017
2360	stretching of C=O	Kalia, 2018
2850	stretch of C–H	Khanna et al., 2017
2920	stretching of C=O	Kalia, 2018
3290	vibration of OH group of cellulose	Jawaid et al., 2017

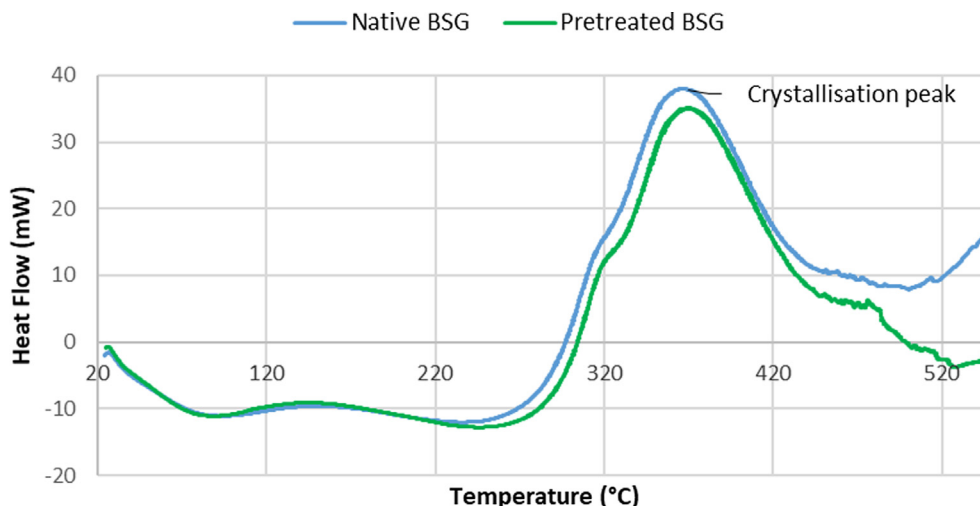


Fig. 4. DSC thermogram of native and pretreated BSG.

Both native and pretreated BSG exhibited a similar trend in their thermo-gram profile, suggesting that they were approximately similar in their composition. An exothermic event was evident between a temperature range of 20–300 °C. This temperature range was associated with several processes which gave rise to compounds such as carbon monoxide, carbon dioxide and other pyrolysis products. In addition, an endothermic event was observed between a temperature range of 300–500 °C. Both native and pretreated BSG exhibited a thermal behaviour that included a crystallization peak at the temperature of 370 °C.

4. Conclusion

The optimal conditions for ultrasound-assisted pretreatment of BSG in aqueous medium were found to be 20% US power, 60 min, 26.3 °C, and 17.3% w/v of biomass. BSG pretreatment under the optimal ultrasonication conditions resulted in a 2.1-fold higher reducing sugar yield, relative to native BSG. These results suggest that ultrasound pretreatment is a viable technology for increased valorization of BSG, and further work is needed in this area.

E-supplementary data of this work can be found in online version of the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to acknowledge the funding from TU Dublin–City Campus (formerly DIT) under the Fiosraigh Scholarship programme, 2017. The authors also wish to acknowledge the funding received from Science Foundation Ireland (16/RC/3889) for the Bioeconomy Research Centre.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2020.02.012>.

References

- Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I., 2014. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. *Food Bioprocess Technol.* 7, 3493–3503. <https://doi.org/10.1007/s11947-014-1349-z>.
- BOE (The Brewers of Europe), 2017. Beer Statistics 2017 edition. Brussels.
- Bundhoo, Z.M.A., Mohee, R., 2018. Ultrasound-assisted biological conversion of biomass and waste materials to biofuels: A review. *Ultrason. Sonochem.* 40 A, 298–313. <https://doi.org/10.1016/j.ultsonch.2017.07.025>.
- Bussemaker, M.J., Zhang, D., 2013. Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications. *Ind. Eng. Chem. Res.* 52, 3563–3580. <https://doi.org/10.1021/ie3022785>.
- Coletti, A., Valerio, A., Vismara, E., 2013. Posidonia oceanica as a renewable lignocellulosic biomass for the synthesis of cellulose acetate and glycidyl methacrylate grafted cellulose. *Materials (Basel)*. 6, 2043–2058. <https://doi.org/10.3390/ma6052043>.
- Eblaghi, M., Niakousari, M., Sarshar, M., Mesbahi, G.R., 2016. Combining ultrasound with mild alkaline solutions as an effective pretreatment to boost the release of sugar trapped in sugarcane bagasse for bioethanol production. *J. Food Process Eng.* 39, 273–282. <https://doi.org/10.1111/jfpe.12220>.
- Gabhane, J., William, S.P., Narayan Vaidya, A., Anand, D., Wate, S., 2014. Pretreatment of garden biomass by alkali-assisted ultrasonication: effects on enzymatic hydrolysis and ultrastructural changes. *J. Environ. Health Sci. Eng.* <https://doi.org/10.1186/2052-336X-12-76>.
- Flores-Gómez, C.A., Escamilla Silva, E.M., Zhong, C., Dale, B.E., da Costa Sousa, L., Balan, V., 2018. Conversion of lignocellulosic agave residues into liquid biofuels using an AFEX™-based biorefinery. *Biotechnol. Biofuels* 11, 7. <https://doi.org/10.1186/s13068-017-0995-6>.
- Hassan, S.S., Williams, G.A., Jaiswal, A.K., 2018a. Emerging Technologies for the Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* 262, 310–318. <https://doi.org/10.1016/j.biortech.2018.04.099>.
- Hassan, S.S., Williams, G.A., Jaiswal, A.K., 2018b. Lignocellulosic Biorefineries in Europe: Current State and Prospects. *Trends Biotechnol.* <https://doi.org/10.1016/j.tibtech.2018.07.002>.
- Hassan, S.S., Williams, G.A., Jaiswal, A.K., 2019. Moving towards the second generation of lignocellulosic biorefineries in the EU: Drivers, challenges, and opportunities. *Renew. Sustain. Energy Rev.* 101, 590–599.
- He, Z., Wang, Z., Zhao, Z., Yi, S., Mu, J., Wang, X., 2017. Influence of ultrasound pretreatment on wood physiochemical structure. *Ultrason. Sonochem.* 34, 136–141. <https://doi.org/10.1016/j.ultsonch.2016.05.035>.
- Jawaid, M., Tahir, P.M., Saba, N., 2017. Lignocellulosic fibre and biomass-based composite materials : processing, properties and applications. Woodhead Publishing.
- Kalia, S., 2018. Lignocellulosic composite materials. Springer Series on Polymer and Composite Materials.
- Karimi, M., Jenkins, B., Stroeve, P., 2014. Ultrasound irradiation in the production of ethanol from biomass Ultrasound irradiation in the production of ethanol from biomass. *Renew. Sustain. Energy Rev.* 40, 400–421. <https://doi.org/10.1016/j.rser.2014.07.151>.
- Karta, M., Depci, T., Karaca, H., Onal, M., Ali Coskun, M., 2016. Co-liquefaction Behaviour of Elbistan Lignite and Olive Bagasse. *Environmental Science*.
- Kartel, M., Galysh, V., 2017. New Composite Sorbents For Caesium And Strontium Ions Sorption. *Chem. J. Mold. Gen. Ind. Ecol. Chem.* 12, 37–44. <https://doi.org/10.19261/cjm.2017.401>.
- Khanna, R., Ikram-UI-Haq, M., Rawal, A., Rajarao, R., Sahajwalla, V., Cayumil, R., Mukherjee, P.S., 2017. Formation of carbyne-like materials during low

- temperature pyrolysis of lignocellulosic biomass: A natural resource of linear sp carbons. *Sci. Rep.* 7, 16832. <https://doi.org/10.1038/s41598-017-17240-1>.
- Kumar, R.S., Shankar, T., Anandapandian, K.T.K., 2011. Characterization of alcohol resistant yeast *Saccharomyces cerevisiae* isolated from Toddy. *Int. Res. J. Microbiol. (IRJM)* 2 (10), 399–405.
- Lynch, K.M., Steffen, E.J., Arendt, E.K., 2016. Brewers' spent grain: a review with an emphasis on food and health. *J. Inst. Brew.* 122, 553–568. <https://doi.org/10.1002/jib.363>.
- Manorach, K., Poonsrisawat, A., Viriya-empikul, N., Laosiripojana, N., 2015. Optimization of sub-critical water pretreatment for enzymatic hydrolysis of sugarcane bagasse. *Energy Procedia* 79, 937–942. <https://doi.org/10.1016/j.egypro.2015.11.590>.
- McCarthy, A.L., O'Callaghan, Y.C., Piggott, C.O., FitzGerald, R.J., O'Brien, N.M., 2013. Brewers' spent grain; bioactivity of phenolic component, its role in animal nutrition and potential for incorporation in functional foods: a review. *Proc. Nutr. Soc.* 72, 117–125. <https://doi.org/10.1017/S0029665112002820>.
- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426–428. <https://doi.org/10.1021/ac60147a030>.
- Mussatto, S.I., 2014. Brewer's spent grain: a valuable feedstock for industrial applications. *J. Sci. Food Agric.* 94 (7), 1264–1275. <https://doi.org/10.1002/jsfa.6486>.
- Peng, W., Wang, L., Ohkoshi, M., Zhang, M., 2015. Separation of hemicelluloses from Eucalyptus species: Investigating the residue after alkaline treatment. *Cellul. Chem. Technol.* 49, 241–244. <https://doi.org/10.1016/j.jcsps.2016.04.005>.
- Plaza, P.E., Gallego-Morales, L.J., Peñuela-Vásquez, M., Lucas, S., Teresa García-Cubero, M., Coca, M., 2017. Biobutanol production from brewer's spent grain hydrolysates by *Clostridium beijerinckii*. *Bioresour. Technol.* <https://doi.org/10.1016/j.biortech.2017.07.139>.
- Raghavi, S., Sindhu, R., Binod, P., Gnansounou, E., Pandey, A., 2016. Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. *Bioresour. Technol.* 199, 202–210. <https://doi.org/10.1016/j.biortech.2015.08.062>.
- Raftopoulou, E., 2019. The Suitability of Brewers' Spent Grain (BSG) for 2nd Generation Bioethanol Production: A Combined Biochemical and Economic Assessment. University of Piraeus.
- Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K., 2018. A comparative analysis of pretreatment strategies on the properties and hydrolysis of brewers' spent grain. *Bioresour. Technol.* 248, 272–279. <https://doi.org/10.1016/j.biortech.2017.06.039>.
- Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K., 2017a. Evaluation of ultrasound assisted potassium permanganate pre-treatment of spent coffee waste. *Bioresour. Technol.* 224, 680–687. <https://doi.org/10.1016/j.biortech.2016.11.034>.
- Ravindran, R., Sarangapani, C., Jaiswal, S., Cullen, P.J., Jaiswal, A.K., 2017b. Ferric chloride assisted plasma pretreatment of lignocellulose. *Bioresour. Technol.* 243, 327–334. <https://doi.org/10.1016/j.biortech.2017.06.123>.
- Rehman, M.S.U., Kim, I., Kim, K.H., Han, J.-I., 2014. Optimization of sono-assisted dilute sulfuric acid process for simultaneous pretreatment and saccharification of rice straw. *Int. J. Environ. Sci. Technol.* 11, 543–550. <https://doi.org/10.1007/s13762-013-0294-0>.
- Rojas-Chamorro, J.A., Cara, C., Romero, I., Ruiz, E., Romero-García, J.M., Mussatto, S.I., Castro, E., 2018. Ethanol production from brewers' spent grain pretreated by dilute phosphoric acid. *Energy Fuels* 32, 5226–5233. <https://doi.org/10.1021/acs.energyfuels.8b00343>.
- Rojas-Chamorro, J.A., Romero, I., López-Linares, J.C., Castro, E., 2019. Brewer's Spent Grain As A Source Of Renewable Fuel Through Optimized Dilute Acid Pretreatment. *Renew. Energy* In press. <https://doi.org/10.1016/j.renene.2019.12.030>.
- Sasmal, S., Goud, V.V., Mohanty, K., 2012. Ultrasound Assisted Lime Pretreatment of Lignocellulosic Biomass toward Bioethanol Production. *Energy Fuels* 26, 3777–3784. <https://doi.org/10.1021/ef300669w>.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of Structural Carbohydrates and Lignin in Biomass: Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory, USA.
- Song, X., Zhang, M., Pei, Z.J., Wang, D., 2015. Ultrasonic-Assisted Dilute Acid Pretreatment of Poplar Wood Biomass for Biofuel Manufacturing: A Preliminary Study. In: Volume 1: Processing. ASME, p. V001T02A074. <https://doi.org/10.1115/MSEC2015-9340>.
- Subhedar, P.B., Gogate, P.R., 2013. Alkaline and ultrasound assisted alkaline pretreatment for intensification of delignification process from sustainable raw-material. *Ultrason. Sonochem. J.* 216–225. <https://doi.org/10.1016/j.ultsonch.2013.08.001>.
- Suresh, K., Ranjan, A., Singh, S., Moholkar, V.S., 2014. Mechanistic investigations in sono-hybrid techniques for rice straw pretreatment. *Ultrason. Sonochem.* 21 (1), 200–207. <https://doi.org/10.1016/j.ultsonch.2013.07.010>.
- Tang, S.Y., Sivakumar, M., 2015. Ultrasound as a Green Processing Technology for Pretreatment and Conversion of Biomass into Biofuels. Springer, Dordrecht, pp. 189–207.
- Terán Hilaes, R., Ramos, L., da Silva, S.S., Dragone, G., Mussatto, S.I., dos Santos, J.C., 2018. Hydrodynamic cavitation as a strategy to enhance the efficiency of lignocellulosic biomass pretreatment. *Crit. Rev. Biotechnol.* 38, 483–493. <https://doi.org/10.1080/07388551.2017.1369932>.
- Rojas-Chamorro, J.A., Romero, I., Ruiz, E., Cara, C., Castro, E., 2017. Comparison of Fermentation Strategies for Ethanol Production from Pretreated Brewers Spent Grain. *Chem. Eng. Trans.* <https://doi.org/10.3303/CET1761104>.
- Wang, Z., Hou, X., Sun, J., Li, M., Chen, Z., Gao, Z., 2018. Comparison of ultrasound-assisted ionic liquid and alkaline pretreatment of Eucalyptus for enhancing enzymatic saccharification. *Bioresour. Technol.* 254, 145–150. <https://doi.org/10.1016/j.biortech.2018.01.021>.
- Wilkinson, S., Smart, K.A., James, S., Cook, D.J., 2017. Bioethanol Production from Brewers Spent Grains Using a Fungal Consolidated Bioprocessing (CBP) Approach. *BioEnergy Res.* 10, 146–157. <https://doi.org/10.1007/s12155-016-9782-7>.
- Xiros, C., Topakas, E., Katapodis, P., Christakopoulos, P., 2008. Evaluation of *Fusarium oxysporum* as an enzyme factory for the hydrolysis of brewer's spent grain with improved biodegradability for ethanol production. *Ind. Crops Prod.* 28, 213–224. <https://doi.org/10.1016/j.indcrop.2008.02.004>.